JOURNAL OF BACTERIOLOGY, Sept. 1997, p. 5670–5676 Vol. 179, No. 18
0021-9193/97/$04.00+0


# *capA*, a *cspA*-Like Gene That Encodes a Cold Acclimation Protein in the Psychrotrophic Bacterium *Arthrobacter globiformis* SI55

FRANÇOISE BERGER, PHILIPPE NORMAND, AND PATRICK POTIER*

*Laboratoire d'Écologie Microbienne du Sol, UMR 5557, Université Claude Bernard, Lyon I, 69622 Villeurbanne Cedex, France*

Received 6 March 1997/Accepted 2 July 1997

**By use of *Arthrobacter globiformis* SI55, a psychrotrophic bacterium capable of growth between −5 and +32°C, we cloned and sequenced *capA*, a gene homologous to *cspA* encoding the major cold shock protein in *Escherichia coli*. The deduced protein sequence has a high level of identity with the sequences of other CspA-related proteins from various sources, and no particular residue or domain that could be specific to cold-adapted microorganisms emerged. We show that CapA was produced very rapidly following cold shock, but unlike its mesophilic counterparts, it was still expressed during prolonged growth at low temperature. Its synthesis is regulated at the translational level, and we showed that growth resumption following a temperature downshift correlated with CapA expression. Transient inhibitions in protein synthesis during the first stages of the cold shock response severely impaired the subsequent acclimation of *A. globiformis* SI55 to low temperature and delayed CapA expression. The cold shock response in *A. globiformis* SI55 is an adaptative process in which CapA may play a crucial role. We suggest that low-temperature acclimation is conditioned mainly by the ability of cells to restore an active translational machinery after cold shock in a process that may be different from that present in mesophiles.**

Recent interest has focused on the effects of low temperatures on the protein content of various microorganisms. Interestingly, bacteria as well as eukaryotic cells respond to cold shock treatments by overexpressing a specific set of proteins, the cold shock proteins or Csps, suggesting that a universal cold shock regulon exists (see reference 19 for a review). The most thoroughly studied response to hypothermic stress is that of *Escherichia coli*. An abrupt shift in growth temperature from 37 to 10°C causes the cessation of growth for 4 to 5 h concomitant with a severe reduction in the number of proteins synthesized. During this lag period, the relative synthesis of a set of Csps is transiently increased. These Csps appear to be involved in various cellular functions such as transcription, translation, and DNA recombination (22). CspA has been identified as the major cold shock protein, and its synthesis is transiently induced, with up to 13% of total cellular protein synthesis attained following the temperature downshift (13). CspA binds to single-stranded nucleic acids (24), and its possible function as an RNA chaperone has been suggested (19). Another function of CspA is as a transcriptional enhancer of at least two other cold shock genes, *hns*, encoding the major nucleoid protein H-NS (25), and *gyrA*, encoding the A subunit of DNA gyrase (21). Previous reports suggested that the transient overexpression of CspA during the early stages of the cold shock response is regulated at the level of transcription (18, 19). However, recent evidence supports a mechanism in which posttranscriptional regulation plays a crucial role in determining the level of CspA in the cell: the *cspA* mRNA has an extremely short half-life at 37°C and is drastically stabilized after a shift from 37 to 15°C (5, 12). However, in spite of these recent advances, the in vivo physiological implication of CspA remains unclear: it belongs to a redundant family of seven homologous proteins (CspA to CspG) in which only CspA, CspB, and CspG are transiently overexpressed following cold shock (13, 26, 29, 37). It has been suggested that CspA, CspB, and CspG may constitute a subfamily whose functions might be closely related to each other but distinctive from the others (29). The relation between the transient induction of CspA, CspB, and CspG following cold shock and the particular involvement of these proteins in the physiology of cells at low temperature and/or in protection of cells from damages due to cold has not yet been clearly demonstrated.

Psychrotrophs and psychrophiles belong to extremely diverse genera, and in addition to specific mechanisms developed during the course of evolution, adaptation to identical thermic constraints may imply common molecular bases that allow the maintenance of vital cellular functions at low temperatures. It may be expected that particular mechanisms exist in the process of acclimation to cold that allow psychrophiles and psychrotrophs to cope with temperatures near or below freezing, whereas mesophiles cannot. In this respect, studies on extremophiles may therefore provide new insights on the molecular strategies adopted in response to stress such as those induced by extremes of temperature. Recently, a cold shock response was described for *Arthrobacter globiformis* SI55, a psychrotrophic bacterium capable of growth between −5 and +32°C (3). One of the cold-regulated proteins (protein A9) was identified as the product of a gene that may be homologous to the *cspA* gene of *E. coli*. The regulation of its expression, however, is different from that of its counterparts in mesophiles since it is still synthesized in cells growing at low temperature. Therefore, it belongs to the particular class of cold acclimation proteins (Caps) defined by Hebraud et al. (15) and Berger et al. (3) which has been described to date only for cold-adapted microorganisms.

In this work, we have cloned and determined the entire

* Corresponding author. Mailing address: Laboratoire d'Ecologie Microbienne du Sol, UMR 5557, Université Claude Bernard, Lyon I, 43 bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France. Phone: 33 4 72 43 13 78. Fax: 33 4 72 43 12 23. E-mail: potier@biomserv.univ-lyon1.fr.

nucleotide sequence of *capA*, a gene encoding a protein of the CspA family in the psychrotrophic bacterium *A. globiformis* SI55. The amino acid sequence of the corresponding protein (CapA) was compared with that of other CspA-related proteins from various sources. We show that the cold shock response in *A. globiformis* SI55 is an adaptative process that enables cells to protect themselves from the deleterious effects of cold. Further, growth resumption at low temperature following cold shock is correlated with CapA synthesis. The regulation of CapA expression is at the translational level, and our results provide indirect evidence that its synthesis is conditioned by the ability of cells to restore an efficient translational machinery after cold shock.

## MATERIALS AND METHODS

**Bacterial strains and growth conditions.** *A. globiformis* SI55 was grown aerobically at 25°C in liquid complete medium or in liquid synthetic medium as described previously (3). *E. coli* DH5α competent cells (Gibco-BRL, Gaithersburg, Md.) were grown at 37°C in Luria broth and used as the host strain for transformation.

**Cloning and sequencing.** All cloning methods were carried out as described by Maniatis et al. (27) and in the manufacturer's instructions. *A. globiformis* SI55 genomic DNA was prepared as described previously (3). The DNA was digested with different restriction enzymes, including *Bam*HI, *Eco*RI, *Kpn*I, *Bcl*I, *Cla*I, *Bgl*II, *Xho*I, *Ksp*I, *Sac*I, *Pst*I, and *Sal*I, separated on a 0.8% (wt/vol) agarose gel, transferred to a nitrocellulose filter, and hybridized with the [$\gamma$-$^{32}$P]ATP-radiolabelled probe FGP-371 (5′-TTCAACGCTGAAAAGGGCTTCGGCTTCAT CA-3′). Cleaved chromosomal DNA fragments, with molecular weights similar to that of a fragment which hybridized with the probe, were ligated into a pBluescript SK$^-$ vector (Stratagene, LaJolla, Calif.). DNA was sequenced with a T7 sequencing kit (Pharmacia LKB, Uppsala, Sweden).

**Sequence data analysis.** GenBank was scanned for related sequences by use of the algorithm BLAST (1). Sequences were aligned with CLUSTAL (16). Clustering analysis was with the neighbor-joining (33) and parsimony (9) methods. The robustness of the distance tree was assessed by the bootstrap method (9). Graphic representation of the resulting tree was made with the NJPlot software of M. Gouy (Laboratoire de Biométrie, U.M.R. C.N.R.S. 5558, Université Lyon I).

The amino acid sequence of CapA was analyzed with the computer program MacVector 3.0 for the calculation of isoelectric point (pI) and molecular weight and for the prediction of the secondary structure of the protein.

**Inhibition of protein synthesis during cold shock.** *A. globiformis* SI55 cells were grown in complete medium at 25°C (optimal growth temperature) to the mid-exponential phase (optical density at 570 nm, 0.5) and treated with 600 μg of chloramphenicol ml$^{-1}$ for 15 min. At this concentration, growth and amino acid incorporation into proteins are totally inhibited in *A. globiformis* SI55 (31) and CapA synthesis is not induced (3). The culture was then divided into four identical aliquots (50 ml). Each fraction was shifted to 4°C and incubated at this temperature in the presence of the antibiotic for 1, 2, 4, or 6 h. Chloramphenicol was then removed by washing the cells twice with cold fresh medium, and the pellets were resuspended in precooled fresh medium to an optical density of 0.2 at 570 mn. Adaptation to 4°C of cold-shocked cells was then assayed by monitoring subsequent growth at 570 nm. At set times, aliquots of cells were harvested by centrifugation (12,000 × *g* for 5 min) for the immunodetection of CapA.

Control experiments were performed by treating exponentially growing cells shifted from 25 to 4°C exactly as described for chloramphenicol-treated cells (including washings) except that the antibiotic treatment was omitted. The efficiency of chloramphenicol removal was tested by treating exponentially growing cells at 4°C with chloramphenicol for the same periods of time, washing the cells twice with precooled fresh medium, and monitoring subsequent growth at 4°C as described above.

**Inhibition of transcription during cold shock.** *A. globiformis* SI55 cells growing exponentially at 25°C in liquid synthetic medium were treated with rifampin (200 μg · ml$^{-1}$) for 30 min. These conditions were found to totally inhibit radioactive uracil incorporation into RNA and resulted in a 95% protein synthesis inhibition as measured by incorporation of radioactive methionine (data not shown). The culture was then shifted to 4°C in the presence of the antibiotic for 4 h. Proteins were radiolabelled by adding 10.25 μCi (3.75 MBq) of L-[$^{35}$S]methionine (specific activity, 42.2 TBq mmol$^{-1}$) for the last 30 min of incubation at 4°C. Cells were rapidly harvested by centrifugation into precooled Eppendorf tubes, and methionine incorporation was stopped by adding 1 ml of cold acetone to the cell pellet. The cells were resuspended into 200 μl of O'Farrell (30) lysis buffer, and cell walls were disrupted by sonication at 50 W (Braun Labsonic) for 8 min (30-s periods). Unbroken cells were removed by centrifugation (12,000 × *g* for 5 min). Total protein extracts were stored at −80°C. In control experiments, cells were treated exactly as described for rifampin-treated cells, except that the antibiotic treatment was omitted.

Total proteins of rifampin-treated and control cells were separated by two-dimensional electrophoresis as described previously (3). The pH gradient along the first-dimension gels was monitored by the use of two-dimensional electrophoresis standards (Bio-Rad S.A., Ivry-sur-Seine, France). Molecular weights were estimated by the comigration of standard proteins (Bio-Rad) in the second dimension. Identification of CapA upon two-dimensional gel electrophoresis was based on its apparent molecular mass (9 kDa) and its isoelectric point (4.5) and was confirmed by immunoblotting.

**Immunodetection of CapA.** In a previous work (3), the sequence of a portion of the *capA* gene was determined with a PCR strategy based on sequence similarity with previously published sequences from *E. coli* and *Bacillus subtilis*. A 14-residue synthetic peptide (Lys-Gly-Phe-Gly-Phe-Ile-Thr-Pro-Asp-Asp-Ser-Asp-Gly-Asp) corresponding to the *A. globiformis* SI55 peptide sequence deduced from the nucleotide sequence was used to produce a polyclonal antibody which allowed the detection of CapA by immunoblotting two- or one-dimensional electrophoresis gels of *A. globiformis* SI55 total proteins (3). Thus, when indicated, *A. globiformis* SI55 cells were harvested by centrifugation (12,000 × *g* for 5 min). The pellets were resuspended in cold acetone and stored for 24 h at −20°C. Acetone was removed by centrifugation (8,000 × *g* for 10 min), and the pellets were dried under vacuum. Cells were washed twice with TKM (50 mM Tris-HCl [pH 7.6], 10 mM $MgCl_2$, 50 mM KCl) and then resuspended in the same buffer. Protein extracts were obtained as described above. The protein concentration in the samples was determined by the method of Bradford (4). Total proteins were separated by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 15% (wt/vol) polyacrylamide gels or by two-dimensional electrophoresis. Sixty and 300 μg of proteins from each sample were loaded for SDS-PAGE and two-dimensional electrophoresis, respectively. Expression of CapA was detected after electroblotting with the polyclonal antibody as described previously (3).

## RESULTS

**Cloning and sequencing of *capA*, a gene encoding a CspA-like protein in *A. globiformis* SI55.** Southern analysis of *A. globiformis* SI55 genomic DNA with the DNA probe FGP-371 complementary to the *cspA*-like gene (3) revealed one major band and one minor band in each digest except those with *Eco*RI and *Bgl*II (data not shown). The (major) 1.7-kb band generated by *Sal*I digestion and the (minor) 4.0-kb generated by *Xho*I were cloned into pBluescript SK$^-$ vector and sequenced.

One clone containing the 4.0-kb insert was found to be homologous to *rpoB* of *E. coli* (GenBank accession no. U76222). This plasmid was not considered further in the present work. Another clone contained a plasmid with a 1.7-kb insert which was designated pFB1, the sequence of which was determined and deposited into GenBank (accession no. L41167). Only two open reading frames (ORFs) were found to have a high probability of coding for a gene according to Fickett's algorithm (10). The ORF beginning beyond the first nucleotide and ending at coordinate 504 is highly similar to a sequence present in *Mycobacterium leprae* and *Mycobacterium tuberculosis* (GenBank accession no. Z73902) with an unknown function. The other ORF begins with the usual start codon ATG at coordinate 817 and ends 201 nucleotides downstream with a TAG termination codon (coordinate 1018). A potential ribosome binding site (GAGGAG) is located 6 nucleotides upstream of the start codon. Two inverted repeat sequences extending 43 nucleotides downstream of the end codon and followed by a string of six T's constitute a putative *rho*-independent terminator (data not shown). No similarity was detected with the sequences downstream of the terminator sequence. The 1.022-kb fragment extending from nucleotides 488 to 1510 and containing the second ORF was subcloned into pBR328. The resulting plasmid was named pPC1.

**Protein sequence comparison of CapA and other CspA-related proteins.** The calculated pI and molecular mass of CapA are 4.503 and 7.137 kDa, respectively, and correspond to the previously described protein A9 coordinates identified by two-dimensional gel electrophoresis (3). According to a secondary-structure prediction by the methods of Chou and Fassman (6) and Garnier et al. (11), an alpha-helical conformation seems


FIG. 1. Clustering (neighbor-joining) analysis of CapA and all homologous bacterial proteins known to date. *, homologous proteins from psychrophilic or psychrotolerant bacteria; #, homologous proteins from thermophilic bacteria; **, homologous proteins from mesophilic bacteria. The bootstrap results above 95% are shown above nodes supported by subsampling analysis, and the dollar signs below nodes indicate those also found by parsimony analysis. Bar, 0.081 substitution/site.

unlikely. The peptide sequence was compared to that in GenBank (National Center for Biotechnology Information) with the algorithm BLASTP (1). The most similar protein sequences were those of *E. coli* cold shock-like proteins CspE and CspA (72 and 69% identity, respectively). The lowest percentages of identity, 37, 48, and 54%, were with *E. coli* CspF, *Haemophilus influenzae* CspD, and *E. coli* CspD, respectively (data not shown). This result confirms that this peptide is a CspA-like protein and corresponds to the previously identified protein A9 in *A. globiformis* SI55 (3). Since it is also overexpressed in 4°C-adapted cells, it belongs to group III of early cold acclimation proteins defined by Berger et al. (3). Therefore, we named it CapA. The analysis of the relationships between CapA and all homologous proteins known to date shows that the sequence of CapA from *A. globiformis* SI55 does not belong to any well-defined cluster of sequences (Fig. 1). The groupings obtained from the clustering analyses performed are not congruent with the 16S-based phylogeny of these bacteria, i.e., the sequences of gram-positive high-GC bacteria (*Streptomyces coelicolor* and *Streptomyces clavuligerus*) group with those of *E. coli* and *H. influenzae* CspD, far away from the sequence of their close phylogenetic neighbor *Arthrobacter* spp. This absence of clustering of the sequences of gram-positive high-GC bacteria was found in all analyses performed. Furthermore, the comparison of the primary sequences of homologous proteins in thermophiles, mesophiles, and psychrotrophs did not reveal any particular domain specific to cold-adapted microorganisms.

**Expression of CapA following cold shock.** When *A. globiformis* SI55 cells exponentially growing at 25°C were shifted to 4°C, a lag phase of 14 h was observed before growth resumed with a generation time of 25 h characteristic of this temperature (Fig. 2). The kinetics of CapA appearance following cold shock shows that CapA was detected as soon as 20 min following transfer to the low temperature and was still present 24 h after the shock (Fig. 2) and even after 40 h (data not shown). It appears, therefore, that while CapA expression occurred very rapidly after transfer to the low temperature, it was still present in cold-adapted cells.

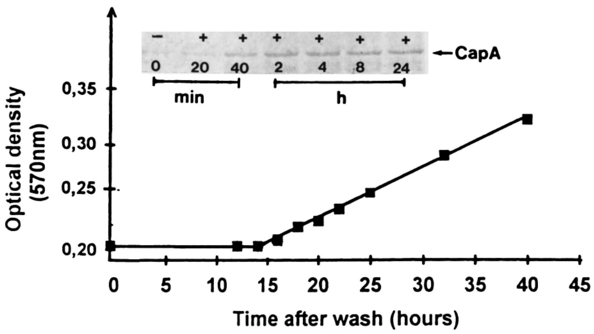


FIG. 2. Growth of *A. globiformis* SI55 following cold shock and kinetics of CapA expression. Exponentially growing cells at 25°C were shifted to 4°C, and subsequent growth was monitored at 570 nm. At the times indicated, aliquots were removed from the culture and total proteins were separated by SDS-PAGE. CapA expression was detected on Western blots as described in Materials and Methods. Signal intensities were estimated visually. −, signal equal to that obtained with 25°C growing cells; +, CapA overexpression.

**Effect of rifampin treatment.** To determine the level of regulation of CapA expression, rifampin was used to inhibit transcription in *A. globiformis* SI55 cold-shocked cells. As shown in the autoradiograms presented in Fig. 3, the expression of most proteins was inhibited but the overexpression of CapA still occurred in cold-shocked cells treated with the antibiotic. This result clearly demonstrates that the mRNA encoding this protein preexisted in cells prior to cold shock, therefore suggesting that CapA expression control is at the translational level.

**Effect of a transient inhibition of the cold shock response on CapA expression and subsequent growth at low temperature.** The hypothesis that the cold shock response in *A. globiformis* SI55 serves an adaptative function in protecting cells from the deleterious effects of cold was then tested. For this, growth at 4°C of cells in which the cold shock response has been transiently inhibited for increasing periods of time was monitored (Fig. 4). When protein synthesis was inhibited with chloramphenicol during the early stages of cold shock, the subsequent kinetics of growth at 4°C were dramatically impaired: there was a delay in growth resumption corresponding to 14 h plus an additional lag (Fig. 4). This additional lag increased with the duration of protein inhibition as follows: +2, +4, +8, and +13 h of lag for chloramphenicol treatments of 1, 2, 4, and 6 h, respectively. We also observed that growth resumed with generation times that significantly increased with the length of protein synthesis inhibition: compared to 25 h for nontreated cells, the generation times observed after 1, 2, 4, and 6 h of chloramphenicol treatment were 36.7, 37.3, 46.3, and 46.3 h, respectively. These effects cannot be due to a deleterious effect of chloramphenicol itself since cells already adapted to 4°C reinitiated growth immediately after chloramphenicol removal (even after 6 h of inhibition) with a generation time of 26 h, which is very similar to that observed for nontreated cells (data not shown). Therefore, our results clearly indicate that the cold shock response is an adaptative process in which protein synthesis is necessary to restore harmonious growth at low temperature.

In parallel, the kinetics of CapA appearance was analyzed by Western blotting (immunoblotting). We observed that the appearance of CapA was delayed for a length of time which increased with the duration of protein inhibition as follows: 2, 6, 8, and 15 h of delay for 1, 2, 4, and 6 h of chloramphenicol treatment, respectively. Therefore, CapA appearance was closely correlated with the duration of the additional lag phase. In all cases, the synthesis of CapA occurred 12 to 14 h before growth resumed.

## DISCUSSION

**CapA of *A. globiformis* SI55 is highly similar to other CspA-related proteins from various origins.** In the psychrotrophic

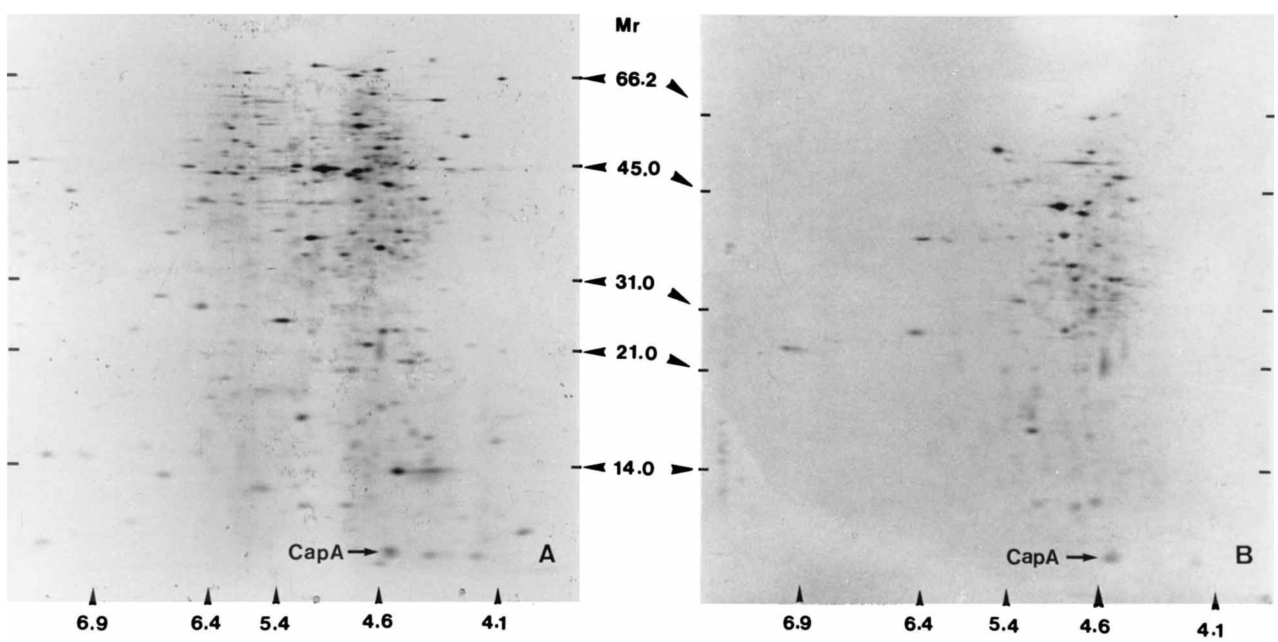


FIG. 3. Effect of rifampin treatment on CapA expression following cold shock. Exponentially growing cells at 25°C were treated with rifampin (200 $\mu g \cdot ml^{-1}$) and shifted to 4°C for 4 h. Cells were labelled with [$^{35}$S]methionine for the last 30 min of incubation at 4°C. Sample preparation and two-dimensional electrophoresis were as described in Materials and Methods. (A) Control cells, no rifampin added; (B) rifampin-treated cells. Isoelectric points (below gels) and molecular weights (in thousands) are indicated. CapA coordinates in two-dimensional gels were determined as described by Berger et al. (3).

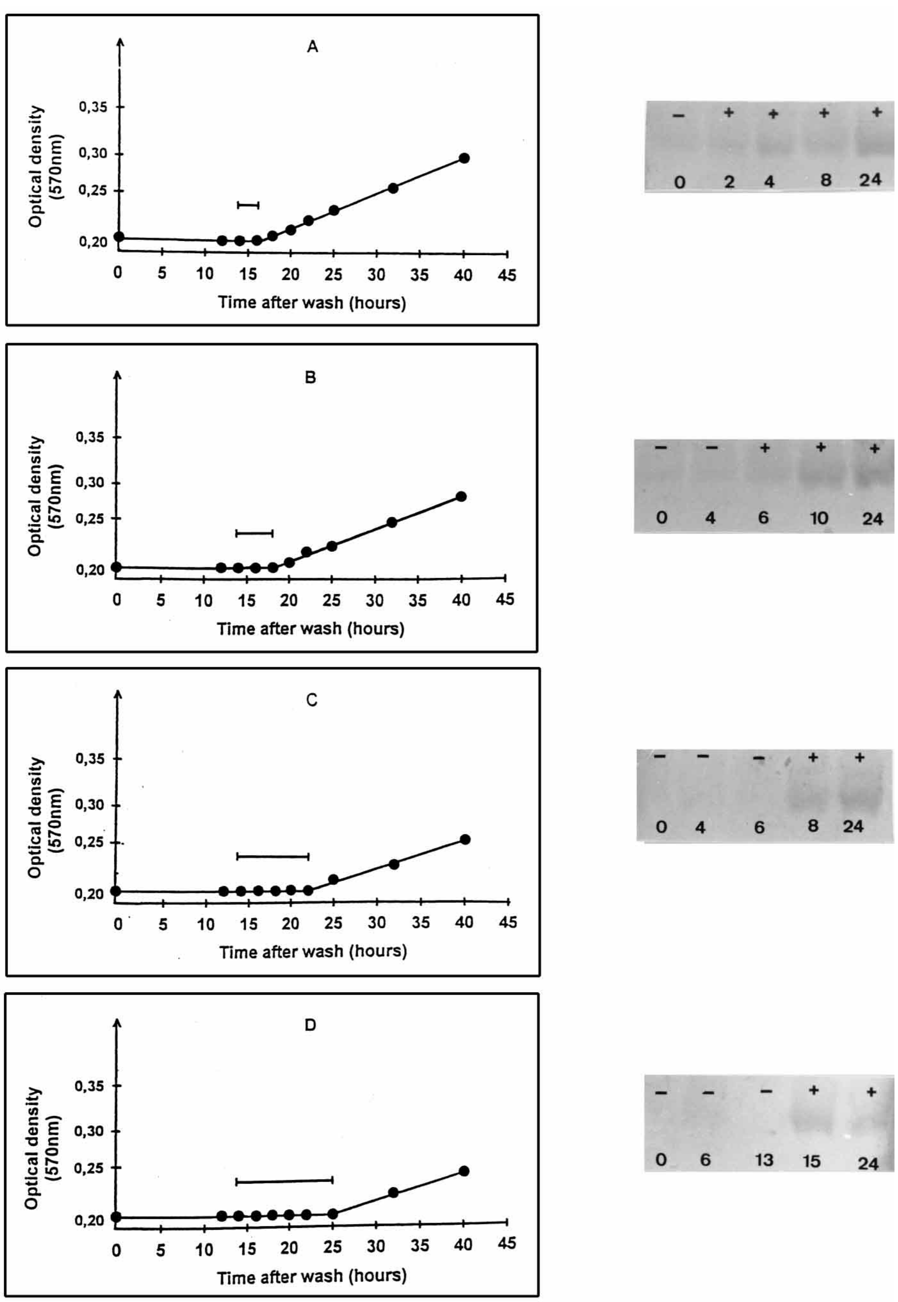


FIG. 4. Effects of chloramphenicol treatments during cold shock on subsequent growth and CapA expression in *A. globiformis* SI55. Chloramphenicol (600 μg · ml$^{-1}$) was added to exponentially growing cells at 25°C. After 15 min of incubation, the cultures were shifted to 4°C. After 1 to 6 h of incubation at this temperature, chloramphenicol was removed and cells were resuspended in fresh medium precooled at 4°C. Subsequent growth and CapA expression were monitored as described in the legend to Fig. 2. Exposure to chloramphenicol was for 1 (A), 2 (B), 4 (C), and 6 (D) h. Bars indicate the lengths of the additional lag phase.

bacterium *A. globiformis* SI55, a portion of a gene that has similarities to *cspA*, the gene encoding the major cold shock protein in *E. coli*, was identified (3). Although several CspA-related proteins from different sources have been described for mesophiles, very few published data on the sequence of similar proteins in cold-adapted bacteria are available (28, 34). We present here the cloning and complete sequencing of this *cspA*-related gene in *A. globiformis* SI55. Since it encodes a protein that is overexpressed following cold shock as well as during prolonged growth at low temperature (Fig. 2), we named this gene *capA* (cold acclimation protein A). The deduced amino acid sequence corresponds to an acidic protein (pI of 4.5) of 67 amino acid residues with a molecular mass of 7,137 Da. These parameters closely match those reported for CspA of *E. coli*. The CapA amino acid sequence has a high level of identity with most members of the CspA family of *E. coli* and with the homologous proteins of other microorganisms. It also contains the highly conserved RNP1 and RNP2 motifs, both domains being involved in the binding to single-stranded DNA and to RNA (24), therefore suggesting related functions of these proteins.

Psychrophilic and psychrotrophic proteins are thought to have evolved toward a structure more flexible than that of their mesophilic or thermophilic counterparts (17). In this respect, we compared the primary sequence of CapA with that of homologous proteins of other psychrotrophs (unpublished or only partially published), mesophiles, and thermophiles (Fig. 1). We found differences between CspA-like protein clustering and the 16S rRNA-based phylogeny. The differences in primary sequence may be due partly to different environmental constraints. This would be particularly evident in such a short gene. However, no relationship could be established between particular residues or domains and bacterial thermic groups. Therefore, which amino acid substitutions are temperature related is difficult to assess, and further Csp-like sequences must be determined before general laws can be proposed.

**CapA is a cold acclimation protein.** We show here that CapA is produced very rapidly (20 min) following a cold shock from 25 to 4°C, demonstrating that *capA* expression is an immediate response to low temperature in *A. globiformis* SI55 (Fig. 2). However, unlike its mesophilic counterparts, the level of expression of CapA remains comparatively low, and it is still detected during prolonged growth at 4°C. The presence of CapA during balanced growth at low temperature could be due to a possible differential stability of this protein at 25 and 4°C. However, previous experiments using radiolabelled amino acid incorporation showed that CapA is actively translated in cells adapted to 4°C (3). Interestingly, a similar expression pattern of CspA-related proteins has also been described for other cold-tolerant microorganisms, such as *Bacillus cereus* (28), *Pseudomonas fragi* (15), and *Listeria monocytogenes* (14). Since this pattern has been observed in all the psychrotrophic bacteria (phylogenetically unrelated) studied to date, it is tempting to suggest that the continuous synthesis of CspA-like proteins during prolonged growth at low temperature is a common feature of cold-adapted microorganisms that distinguishes them from mesophiles. It is believed that the cold shock response serves an adaptative function, and it has been suggested that proteins of the CspA family, together with other Csps, play a role in protecting cells from damage due to cold (13). If this is the case, the continuous overexpression of the proteins of the CspA family at low temperature in psychrotolerant bacteria could contribute to an explanation for why these organisms develop at temperatures near or below freezing whereas mesophiles cannot.

**Growth of *A. globiformis* S155 after cold shock is correlated with CapA synthesis.** Transferring *A. globiformis* SI55 from 25 to 4°C resulted in a 14-h lag, followed by growth with a generation time of 25 h. A temporary block in protein synthesis during the first stages of incubation at the lower temperature resulted in an additional lag that increased with the duration of the inhibition (Fig. 4). Furthermore, growth resumed with generation times that progressively increased with the length of protein synthesis inhibition. Since such effects were not observed in cells already adapted to 4°C, our results clearly demonstrate that the cold shock response in *A. globiformis* SI55 is an adaptative process that enables cells to restore a harmonious physiology at low temperature. We also observed that CapA expression was delayed for periods of time that increased with the duration of protein inhibition. In all cases, CapA synthesis occurred 12 to 14 h before growth resumption at 4°C. Considering that (i) CapA expression occurs very rapidly following temperature downshift, (ii) CapA synthesis involves an active adaptation mechanism, (iii) growth resumption at low temperature is correlated with CapA production, and (iv) CapA is continuously overproduced at low temperature, it is therefore tempting to suggest that CapA plays a central role in the process of cold acclimation in *A. globiformis* S155. To investigate the precise role of this protein at low temperature, the isolation of CapA mutants must be considered. In *B. subtilis*, an insertional inactivation mutant (*cspB*::*cat*) of a gene encoding a CspA-like protein was obtained, and a slight decrease in its viability was demonstrated after cycles of freezing and thawing (36).

**CapA synthesis is regulated at the level of translation.** The results presented in this report demonstrate that CapA synthesis following cold shock is not affected by rifampin treatment in *A. globiformis* SI55 (Fig. 3). This indicates that CapA mRNA preexists in cells prior to cold shock, and posttranscriptional regulation may therefore account for the induction of CapA. Accumulative data indicate that in *E. coli*, posttranscriptional events play a crucial role in determining CspA overexpression following cold shock. It was demonstrated that the *cspA* gene is constitutively transcribed at all temperatures, including 37°C (8). At this temperature, the mRNA of *cspA* is extremely unstable, with a very short half-life of 12 s, so that it cannot be translated. Upon a temperature downshift from 37 to 15°C, the mRNA is drastically stabilized, resulting in efficient translation (5, 8, 12). Hence, it is possible that similar to *E. coli*, CapA expression in *A. globiformis* SI55 may be regulated posttranscriptionally at the level of mRNA stability. However, since a temporary block of the cold shock response delays its synthesis, we suggest that cold-induced modifications of the ribosome are necessary to restore efficient translation at low temperature.

Very recently, Jones and Inouye (20) proposed an interesting cold shock ribosome adaptation model to explain the induction and function of the cold shock response in *E. coli*. They suggest that a shift to low temperature results in a cold-sensitive block in initiation of translation of most cellular mRNAs, whereas mRNAs for the cold shock proteins (including CspA) can be efficiently translated. Cold shock proteins RbfA, CsdA, and initiation factor 2 then associate with ribosomes, resulting in efficient translation of cellular mRNAs. Therefore, as cells adapt to the low temperature, there is an increase in protein synthesis accompanied by repression of the cold shock response.

A cold shock response has been described for several psychrotrophic and psychrophilic microorganisms (2, 3, 7, 15, 23, 28, 32, 35). From these studies, it can be concluded that the overall cold shock responses in all these organisms have the following similarities and are somewhat different from that in *E. coli*. (i) The synthesis of housekeeping proteins is never repressed following an abrupt temperature downshift; most of these proteins are similarly expressed at optimal and low temperatures. (ii) A large number of Csps are synthesized; their number increases (up to 30) with the severity of the shock. (iii) The relative level of expression of the Csps is moderate, even after a severe cold stress. (iv) A second class of cold-related proteins, i.e., the cold acclimation proteins or Caps, which include CspA-related proteins, exists; compared with Csps, the production of Caps does not cease in cells adapted to low temperature. Therefore, the very attractive model of Jones and Inouye (20) can only partially explain the cold shock response in cold-adapted microorganisms, and further studies are necessary to understand how these microorganisms adapt to temperatures near or below freezing.

## ACKNOWLEDGMENT

This work was supported by grant BI04-CT96-0051 (COLDZYME) from the European Community.

## REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.

2. **Araki, T.** 1991. The effect of temperature shifts on protein synthesis by the psychrophilic bacterium *Vibrio* sp. strain ANT-300. J. Gen. Microbiol. **137:** 817–826.
3. **Berger, F., N. Morellet, F. Menu, and P. Potier.** 1996. Cold shock and cold adaptation proteins in the psychrotrophic bacterium *Arthrobacter globiformis* SI55. J. Bacteriol. **178:**2999–3007.
4. **Bradford, H. H.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of proteins using the principle of protein-dye binding. Anal. Chem. **72:**248–254.
5. **Brandi, A., P. Pietroni, C. O. Gualerzi, and C. L. Pon.** 1996. Post-transcriptional regulation of CspA expression in *Escherichia coli*. Mol. Microbiol. **19:**231–240.
6. **Chou, P. Y., and G. D. Fassman.** 1978. Empirical predictions of proteins conformations. Annu. Rev. Biochem. **47:**251–276.
7. **Cloutier, J., D. Prévost, P. Nadeau, and H. Antoun.** 1992. Heat and cold shock protein synthesis in arctic and temperate strains of rhizobia. Appl. Environ. Microbiol. **58:**2846–2853.
8. **Fang, L., W. Jiang, W. Bae, and M. Inouye.** 1997. Promoter-independent cold-shock induction of *cspA* and its derepression at 37°C by mRNA stabilization. Mol. Microbiol. **23:**355–364.
9. **Felsenstein, J.** 1993. PHYLIP (Phylogeny Inference Package), version 3.5c. Department of Genetics, University of Washington, Seattle.
10. **Fickett, J. W.** 1982. Recognition of protein coding regions in DNA sequences. Nucleic Acids Res. **10:**5303–5318.
11. **Garnier, J., D. J. Osguthorpe, and B. Robson.** 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. **120:**97–120.
12. **Goldengerg, D., I. Azar, and A. Oppenheim.** 1996. Differential mRNA stability of the *cspA* gene in the cold shock response of *Escherichia coli*. Mol. Microbiol. **19:**241–248.
13. **Goldstein, J., N. S. Pollitt, and M. Inouye.** 1990. Major cold shock protein of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**283–287.
14. **Hébraud, M.** Personal communication.
15. **Hébraud, M., E. Dubois, P. Potier, and J. Labadie.** 1994. Effect of growth temperatures on the protein levels in a psychrotrophic bacterium *Pseudomonas fragi*. J. Bacteriol. **176:**4017–4024.
16. **Higgins, D. G., and P. M. Sharp.** 1988. CLUSTAL: a package for performing multiple sequence alignments on a microcomputer. Gene **73:**237–244.
17. **Hochachka, P. W., and G. N. Somero.** 1984. Biochemical adaptation. Princeton University Press, Princeton, N.J.
18. **Jiang, W., P. Jones, and M. Inouye.** 1993. Chloramphenicol induces the transcription of the major cold shock gene of *Escherichia coli*: *cspA*. J. Bacteriol. **175:**5824–5828.
19. **Jones, P., and M. Inouye.** 1994. The cold shock response. A hot topic. Mol. Microbiol. **11:**811–818.
20. **Jones, P., and M. Inouye.** 1996. Rbfa, a 30S ribosomal binding factor, is a cold-shock protein whose absence triggers the cold shock response. Mol. Microbiol. **21:**1207–1218.
21. **Jones, P. G., R. Krah, S. R. Tafuri, and A. P. Wolffe.** 1992. DNA gyrase, CS7.4, and the cold shock response in *Escherichia coli*. J. Bacteriol. **174:** 5798–5802.
22. **Jones, P. G., R. A. Van Bogelen, and F. C. Neidhardt.** 1987. Induction of proteins in response to low temperature in *Escherichia coli*. J. Bacteriol. **169:**2092–2095.
23. **Julseth, C. R., and W. E. Inniss.** 1990. Induction of protein synthesis in response to cold shock in the psychrotrophic yeast *Trichosporon pullulans*. Can. J. Microbiol. **36:**519–524.
24. **Landsman, D.** 1992. RNP-1, an RNA-binding motif, is conserved in the DNA-binding cold shock domain. Nucleic Acids Res. **11:**2861–2864.
25. **La Teana, A., A. Brandi, M. Falconi, R. Spurio, C. L. Pon, and C. O. Gualerzi.** 1991. Identification of a cold shock transcriptional enhancer of the *Escherichia coli* gene encoding nucleoid protein H-NS. Proc. Natl. Acad. Sci. USA **88:**10907–10911.
26. **Lee, S. J., A. Xie, W. Jiang, J. P. Etchegaray, P. J. Jones, and M. Inouye.** 1994. Family of the major cold shock protein CspA (CS7,4) of *Escherichia coli*, whose members show a high sequence similarity with the eucaryotic Y-box binding proteins. Mol. Microbiol. **11:**833–839.
27. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Mayr, B., T. Kaplan, S. Lechner, and S. Scherer.** 1996. Identification and purification of a family of dimeric major cold shock protein homologs from the psychrotrophic *Bacillus cereus* WSBC 10201. J. Bacteriol. **178:**2916–2925.
29. **Nakashima, K., K. Kanamaru, T. Mizuno, and K. Horikoshi.** 1996. A novel member of the *cspA* family of genes that is induced by cold shock in *Escherichia coli*. J. Bacteriol. **178:**2994–2997.
30. **O'Farrell, P. H.** 1975. High-resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250:**4007–4021.
31. **Potier, P., A. R. Hipkiss, and D. J. Kushner.** 1985. Protein turnover in a psychrotrophic bacterium. Arch. Microbiol. **142:**28–33.
32. **Roberts, M. E., and W. E. Inniss.** 1992. The synthesis of cold shock proteins and cold acclimation proteins in the psychrotrophic bacterium *Aquaspirillum arcticum*. Curr. Microbiol. **25:**275–278.
33. **Saitou, R. R., and M. Nei.** 1987. A neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **44:**406–425.
34. **Schröder, K., P. Zuber, G. Willimsky, B. Wagner, and M. A. Marahiel.** 1993. Mapping of the *Bacillus subtilis cspB* gene and cloning of its homologs in thermophilic, mesophilic and psychrotrophic bacilli. Gene **136:**277–280.
35. **Whyte, L. G., and W. E. Inniss.** 1992. Cold shock proteins and cold acclimation proteins in a psychrotrophic bacterium. Can. J. Microbiol. **38:**1281–1285.
36. **Willimsky, G., H. Bang, G. Fischer, and M. A. Marahiel.** 1992. Characterization of *cspB*, a *Bacillus subtilis*-inducible cold shock gene affecting cell viability at low temperatures. J. Bacteriol. **174:**6326–6335.
37. **Yamanaka, K., T. Mitani, T. Ogura, H. Niki, and S. Hiraga.** 1994. Cloning, sequencing, and characterization of multicopy suppressors of a *mukB* mutation in *Escherichia coli*. Mol. Microbiol. **13:**301–312.